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Date of Deposit <u>November 21, 2000</u>	Label Number: <u>EJ770213695US</u>
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Substitute Form PTO 1390 U.S. Dept of Commerce Patent and Trademark Office		Attorney's Docket Number: 50199/002001
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application Number: Not Yet Assigned
INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IB99/00924	21 May 1999 (21.05.99)	23 May 1998 (23.05.98)
TITLE OF INVENTION:	IMPROVED PROCESS FOR ALCOHOLIC FERMENTATION	
APPLICANTS FOR DO/EO/US:	JAMES MCLAREN	
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1.	<input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.	
2.	<input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.	
3.	<input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).	
4.	<input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date.	
5.	A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> a. is transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. has been transmitted by the International Bureau. <input type="checkbox"/> c. is not required, as the application was filed with the United States Receiving Office (RO/US).	
6.	<input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).	
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> a. are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. have been transmitted by the International Bureau. <input type="checkbox"/> c. have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> d. have not been made and will not be made.	
8.	<input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).	
9.	<input checked="" type="checkbox"/> An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).	
10.	<input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).	
11.	<input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98	
12.	<input checked="" type="checkbox"/> An assignment for recording. A separate cover sheet in compliance with 37 3.28 and 3.31 is included.	
13.	<input checked="" type="checkbox"/> A FIRST preliminary amendment.	

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	<input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.				
14.	<input type="checkbox"/> A substitute specification.				
15.	<input type="checkbox"/> A change of power of attorney and/or address letter.				
16.	<input checked="" type="checkbox"/> Other items or information: References				
17.	<input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(A)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$ 970.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4) \$ 670.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 96.00				
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130 for furnishing the oath or declaration later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	37 - 20 =	17	x \$18.00	\$306.00	
Independent claims	2 - 3 =	0	x \$80.00	\$	
Multiple dependent claims (if applicable)			+ \$270.00	\$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 1436.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed with this request (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$ 1436.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 1436.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property.				\$ 40.00	
TOTAL FEES ENCLOSED =				\$ 1476.00	
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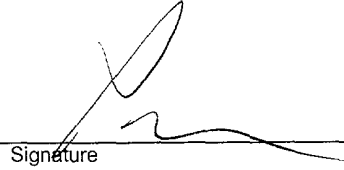
- ☒ a. A check in the amount of \$ 1476.00 to cover the above fees is enclosed.
☐ b. Please charge my Deposit Account No. 03-2095 in the amount of \$ [** **] to cover the above fees.
☒ c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Signature

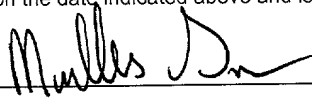
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	James McLaren	Art Unit:	Not Yet Assigned
Serial No.:	Not Yet Assigned	Examiner:	Not Yet Assigned
Filed:	November 21, 2000		
Title:	IMPROVED PROCESS FOR ALCOHOLIC FERMENTATION		

Assistant Commissioner For Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination, kindly amend the above-referenced application as follows.

In the specification

At page 14, line 10, delete "ppm," and insert --mg--.

In the claims

Please cancel claims 1-19, and add new claims 20-39 as follows.

--20. A method for alcoholic fermentation, said method comprising employing in said fermentation:

- a) a fermentation micro-organism; and
- b) at least one mineral-rich or mineral-enriched yeast;

wherein said mineral-rich or mineral-enriched yeast provides a nutrient source for said fermentation.--

--21. The method of claim 20, wherein said mineral-rich or mineral-enriched yeast is selected from the group consisting of the *Saccharomyces* genus and the *Kluyveromyces* genus.--

--22. The method of claims 20 or 21, wherein said mineral-rich or mineral-enriched yeast is obtained by adding 1,000 to 200,000 ppm of a salt of a mineral to a live culture of a yeast, wherein said ppm is measured relative to the dry weight of said yeast, at a temperature of 4°C to 40°C, and at a pH of between 3.5 to 7.0, for a time of 1 hour to 24 hours, wherein said yeast incorporates said mineral.--

--23. The method of claim 22, wherein said temperature is from 25°C to 32°C.--

--24. The method of claim 22, wherein said pH is from 4.6 to 6.6.--

--25. The method of claim 22, wherein said time is from 2 hours to 16 hours.--

--26. The method of claim 22, wherein said salt is selected from the group consisting of acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate, and valerate.--

--27. The method of claim 22, wherein said yeast incorporates said mineral by absorption, adsorption, or both.--

--28. The method of claim 22, wherein said mineral is a metal that is capable of altering the metabolism of said fermentation.--

--29. The method of claim 22, wherein said mineral is selected from the group consisting of zinc, magnesium, and manganese.--

--30. The method of claim 22, wherein the concentration of said mineral in said mineral-rich or mineral-enriched yeast ranges from 1,000 to 200,000 ppm.--

--31. The method of claim 22, wherein said mineral-rich or mineral-enriched yeast is selected from the group consisting of live yeast and dead yeast.--

--32. The method of claim 22, wherein said mineral-rich or mineral-enriched yeast is in a form selected from the group consisting of a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, and a powder.--

--33. The method of claim 22, wherein said mineral-rich or enriched yeast is added directly to at least one step in said fermentation.--

--34. The method of claim 22, wherein said mineral-rich or enriched yeast is added directly to at least one vessel selected from the group consisting of a fermenter, a boiling vessel, any vessel between the two, a fermentation micro-organism-holding vessel, and a fermentation micro-organism propagating vessel.--

--35. The method of claim 22, wherein said yeast is used at such a quantity and/or at such a concentration of said mineral that it leads to an increase of at least 0.05 ppm of the mineral content of the substrate of said fermentation.--

--36. The method of claim 22, wherein said alcoholic fermentation leads to the production of beer.--

--37. The method of claim 22, wherein said alcoholic fermentation is selected from the group consisting of cereal-, fruit-, sugar-, and honey-based fermentation.--

--38. The method of claim 22, wherein said fermentation leads to the production of an alcohol selected from the group consisting of whisky, sake, wine, brandy, cider, fruit wines, mead, rum, tequila, industrial alcohol, and potable alcohol.--


--39. A fermentation composition comprising a fermentation micro-organism and at least one mineral-rich or mineral-enriched yeast.--

Support for the amendments

Support for the amendment to the specification can be found at page 14, line 10, of the specification as originally filed. The claims have been amended to comply with U.S. claim format and to correct typographical errors. No new matter has been added by these amendments. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: Nov. 21, 2000



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5 **IMPROVED PROCESS FOR ALCOHOLIC FERMENTATION**

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The present invention generally relates to an improved process for alcoholic fermentation which comprises the use of a mineral-rich or mineral-enriched yeast as a nutrient in said fermentation process, and to
10 the use of such a yeast as a nutrient in an alcoholic fermentation process.

There are a number of minerals that are required in trace amounts for efficient alcoholic fermentation. These in particular include metals capable of altering the fermentation metabolism, such as divalent metals
15 *e.g.* manganese, magnesium and zinc. There has been an increasing awareness of the importance of such trace minerals in alcoholic fermentations, particularly with respect to beer.

The zinc concentration of a wort is of particular importance from two perspectives. Firstly, if limiting, it can lead to sub-optimal, even
20 incomplete fermentations, problems with head retention and yeast flocculance. Secondly, adequate levels of zinc can aid in the optimisation of alcoholic fermentations, vis a vis ethanol production and fermentable sugars uptake. This second perspective has a greater importance during fermentations when the yeast is subject to greater stresses. Moreover,
25 traditionally, breweries recycle their yeast from one fermentation to another. Repitching yeast from one fermentation where the zinc is limiting

into another beer wort, which is also deficient, would exacerbate fermentation problems.

Other minerals have been shown to be of importance in the course of an alcoholic fermentation. Manganese is thus known to be implicated as a substitute zinc metabolism, and could possibly mitigate some toxic effects associated with high concentrations of zinc. Another example is magnesium which is reported to be important for alcohol efficiency in fermentations. This is particularly a problem for the fermentation of certain substrates where there is an excess of calcium ions present. Calcium is indeed known to be antagonistic to magnesium metabolism and, for example, in beer, calcium is deliberately added in order to control the pH (acidity) and activate some of the enzymes of the malted barley.

For most alcoholic fermentations, there is thus a perceived natural mineral deficit in the substrate, and minerals, in the form of mineral salts such as zinc/manganese/magnesium chloride or sulphate, are generally added directly into the substrate, *e.g.* into the wort at the boiling stage for beer production. The use of such mineral salts, whilst relatively effective, conflicts with the desire by some industrialists to produce additive free alcohols.

Alternatives to the addition of mineral salts have thus been proposed in the past decades. These include pre-loading the fermentation yeast with a metal in such a way that the metal is hardly released from the cell body of the fermentation yeast during the fermentation process (JP 63287474), or using ash trub or acid extracts of spent grains or hop trub (US 4,840,802) so as to make use of the trace elements they contain. But all these alternatives are, in terms of quantity and quality of alcoholic fermentation production, at the best only substantially equivalent to the initial solution of directly adding mineral

salts. Their industrial application is thus quite restricted, and some of them even show problems of off-odours associated with the process (*e.g.* acid extract use). None of the prior art techniques thus provides with fully satisfactory results.

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It is an object of the present invention to provide with an improved process for alcoholic fermentation which, further to not showing the drawbacks of prior art techniques, is quantitatively, and also qualitatively more efficient than the solution of directly adding mineral salts or any other alternative solution. The process and use according to the invention allow an enhanced fermentation yeast growth, and accelerated fermentation. It also shows many advantages : it is very easy to handle, it applies to any alcoholic fermentation process, and is economically very beneficial. The process of the invention comprises the use of at least one mineral-rich or mineral-enriched yeast not as fermentation micro-organism, but as a nutrient source : the present invention indeed shows that, contrary to the received wisdom in the brewing industry, another micro-organism can be efficiently added to a fermentation process without leading to microbial instability, and that it is moreover able to provide the fermentation micro-organism with nutrients, and particularly with minerals such as zinc, magnesium, manganese in a very efficient way. As will be further described and illustrated below, this efficiency as a mineral source not only lies into an efficient mineral flux from the yeast(s) used as a nutrient source towards the fermentation micro-organism : the process and use according to the invention are indeed more efficient than the direct addition into the substrate of an equivalent quantity of mineral salt, and is

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even more efficient than the separate addition of both mineral salt on one hand, and a dead yeast on the other hand (see examples). That is to say, the process according to the invention shows synergetic effects in terms of mineral nutrition. These synergetic effects may at least partially lie in an increased mineral bioavailability favorable to the fermentation micro-organism.

The term "fermentation process" is herein meant as including the whole production process, and is no way limited to the precise biological step of fermentation. It *e.g.* also includes the fermentation yeast propagation step and the process of production of the substrate. The term "nutrient" herein comprises any element which can be considered of nutritive value to the fermentation micro-organism, and thus also comprises micro- or trace nutrients. It has to be also pointed out that the word "yeast" is herein meant as a yeast cell which can be living or dead, and which still comprises at least one structure corresponding to an insoluble cell structure.

A preferred yeast for use according to the invention is a mineral-enriched yeast. Said at least one mineral-rich or mineral-enriched yeast is advantageously chosen among the food grade yeast genera. Examples of appropriate yeasts include the *Saccharomyces* genera (*e.g.* *Saccharomyces cerevisiae*) and the *Kluyveromyces* genera.

In an embodiment of the present invention, said at least one mineral-rich or mineral-enriched yeast is, before use, such as obtained by adding about 1,000 to about 200,000 ppm (relative to the weight of the yeast, as measured on a dry weight basis) of a salt of said mineral to a live culture of yeast at a temperature of about 4-40°C, preferably about 25-32°C, at a pH of between about 3.5 to about 7.0, preferably about 4.6 to 6.6, for a

period of about 1-20 or 1-24 hours, preferably 2-16 hours so as to allow said yeast to incorporate, absorb and/or adsorb the mineral(s). Any salt, e.g. acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide, perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate is appropriate. Comparative assays can be performed by the person skilled in the art to determine the most efficient mineral source. Said mineral incorporation can correspond to an absorption and/or an adsorption. When incorporated, said mineral may remain as a mineral and/or be transformed into a salt and/or an organic form. It has to pointed out that the efficacy of the use according to the invention is not necessarily directly and solely dependent on the resultant mineral concentration of the substrate : bioavailability has also to be taken into account. In another embodiment, said at least one mineral-rich or enriched yeast is a commercially available product, e.g. a product from the Danstar Ferment A.G. Mineral Enriched Yeast range.

Said yeast is advantageously rich in, or enriched in at least one mineral which is capable of altering the metabolism of an alcoholic fermentation. A capacity of altering the metabolism of an alcoholic fermentation can be easily assessed by the person skilled in the art, e.g. by comparing the growth level of the fermentation micro-organism, and/or the rate of fermentation, and/or the secondary metabolites concentrations and/or the flavour profile, in the presence and in the absence of the mineral candidate under standard appropriate laboratory conditions. The word "mineral" herein also comprises oligoelements. Such a mineral is preferably a metal, and most preferably a divalent metal. It is advantageously chosen among the group consisting of zinc, magnesium,

manganese. A most preferred mineral is generally zinc. But when it deals with negating the repressive effect of the calcium and thereby increasing sugar/alcohol conversion, magnesium is then preferred. Said at least one mineral-rich or enriched yeast can carry more than one nutrient mineral at a time, *i.e.* it can be a combination or a permutation of *e.g.* magnesium and zinc.

The use of said at least one mineral-rich or enriched yeast according to the invention is such that the mineral(s) contained therein or thereon is(are) released to the benefit of the fermentative micro-organism culture.

Preferably, said at least one mineral-rich or enriched yeast contains, before being used, a concentration ranging from about 1.000 to about 200.000 ppm for each mineral it carries.

One of the many advantages of the process and use according to the invention lies in the fact that said at least one mineral-rich or enriched yeast can be supplied in any form appropriate to the precise fermentation process wherein it has to be used. It can be supplied in a living form, or in a dead form. It may be cellularly intact, but, as it is used as a nutrient source, and not for a cell production, it also can be cellularly slightly ruptured.

Said at least one mineral-rich or enriched yeast can indeed be used under a variety of forms which include a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, or a powder. It may have been sterilised or not. It may be used on its own or as part of a mixture of other products. The process and use according to the invention can thus be seen as the use of at least one sacrificial yeast as a nutrient source in alcoholic fermentations.

Another advantage lies in the fact that said use can be performed, as desired, at any step of the fermentation process. A simple direct addition of said at least one mineral-rich or enriched yeast at at least one step of the fermentation process is efficient. It may thus be added directly into the boiling vessel, and/or the fermenter, and/or any vessel between the two, and/or into the fermentation micro-organism holding or propagating vessels. For example, in beer production, the addition to the wort can be performed during alcohol production process or fermentation micro-organism propagation process, before or after boiling.

Said at least one mineral-rich or enriched yeast can thus be added directly to the wort so that it is killed during the wort boiling stage. It may be also added to the cooled wort prior to, during or after yeast pitching. Preferably said at least one mineral-rich or enriched yeast is added to the boiling wort. Advantageously, said use according to the invention is performed so that said yeast is used at such a quantity and/or at such a concentration in said mineral that it leads to an increase of at least about 0.05 ppm of the mineral content of the substrate of said fermentation. The fermented substrate itself may be distilled or not.

The use according to the invention is particularly efficient in that it accelerates alcoholic fermentation velocity greater than when the mineral concentration is raised by the addition of the equivalent concentration of mineral when derived from a mineral salt. A synergetic effect can moreover be outlined when comparing to the addition of mineral salt on one hand and dead yeast on the other hand (see e.g. laboratory tests 2 and 3 of example 1 for zinc). The fermentation duration needed therefore decreases (see examples below). The limit to primary fermentation is achieved faster, significantly in comparison to when the equivalent

mineral concentration is derived from a mineral salt. The number of hours necessary to achieve the standard specific gravity (about 3.6°P or about 3.8°P for beer) is decreased: the time needed to achieve the attenuation degree of fermentation is decreased of several hours (about 20 hours in the below examples with zinc).

The use according to the invention also allows the fermentation to progress to absolute dryness, *i.e.* to an absence of residual fermentable sugars in the alcohol thus produced (see *e.g.* the below example 2). It not only allows a higher production of alcohol, but also a qualitatively better one (see *e.g.* the below example 1). And last, but not least, despite said fermentation acceleration, the alcohol produced according to the invention tastes equal to or better, in comparison to when the equivalent mineral concentration is derived from a mineral salt (see also the below example 3).

The process and use according to the present invention are of first interest for the beer industry, but it can also apply to any alcoholic fermentation be it cereal based, such as whisky or sake as well as fruit, sugar or honey based fermentations, such as wine, brandy, cider, fruit wines, mead, rum, tequila, industrial alcohols, potable alcohols, vodka, gin, etc.

The present invention also relates to the use of at least one mineral-rich or mineral-enriched yeast as a nutrient source as herein described for the production of alcohol by a fermentation process.

Technical features and advantages of the present invention are herein further illustrated by several examples, which are given for illustration purposes and are in no way intended in restricting the scope of the invention. In these examples, reference is made to :

- figure 1 which represents the results of brewery trial 1 (Extracts [°Balling] as a function of the number of fermentation days), and to

- figure 2 which represents, in a similar way as for figure 1, the results of brewery trial 2.

5 In figures 1 and 2, the legend is the following :

lozenges : O-wort

squares : zinc yeast

horizontal line : primary attenuation limit

10 **EXAMPLE 1: FERMENTATION SPEED**

Three laboratory tests and two brewery trials were carried out to show the relative effectiveness of sacrificial zinc yeast and zinc chloride addition to worts containing different natural concentrations of zinc, and then fermented by yeasts containing different natural concentrations of zinc.

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Materials and Methods

Materials

20 **Yeast strains and provenance (laboratory tests)**

In the results herein reported, the yeast sources were as follows :

The yeast strain used in all laboratory trials was a strain of *S. cerevisiae* (lager type) obtained from four different commercial breweries in
25 Germany. The sample taken was from their stock designated to be used for their next fermentation. The strain is for tests one, two and three, and

in brewery trial 2 is deposited with the Technical University of Munich-Weihenstephan Hefebank, and designated as strain number W 34/70. The strain used in brewery trial 1 is deposited with the Technical University of Munich-Weihenstephan Hefebank, and designated as strain number W 120.

The yeast used for all laboratory trials was obtained, when needed, in the form of a cream from the appropriate brewery. The cream was centrifuged in a SORVAL® RC5B centrifuge at 2700g for 10 minutes and the supernatant was discarded. The yeast paste was weighed and resuspended in cooled wort, aerated and then added directly to the fresh worts. The zinc content of the yeast was measured before pitching.

Media

Laboratory Test Fermentations

The wort used was obtained from three commercial breweries in Germany. In test number one the wort is used to make their "helle" type beer. In test number two the wort is used to make their "festbier". In test number three the wort is used to produce "pilsner" type beer. In all cases the wort was collected at the end of the boil and was therefore hopped to the normal level of that product for that particular beer. The worts had not been treated in the respective brewery in any way to alter the natural level of zinc. The worts were boiled for fifteen minutes before being cooled to the fermentation temperature, 10°C, and pitched with fermentation yeast.

Brewery Trials

Two brewery trials were carried out in two commercial breweries in Germany. The brewery in trial one was the Brauerei Kreiger, 944505 Landau, D. Isar, Bavaria, Germany. During a normal commercial production phase, two consecutive "helle" type worts, number 22 and 23, produced on 20th and 21st of April 1999 respectively, were designated for experimental observation. They were produced from the same recipe, one immediately after the other, from the same malt and hop stocks, and brewing water. 110 hectolitres of wort was collected from each brew.

The brewery in trial two was, the Privatbrauerei Kitzmann, Kitzmann Bräu KG, Südliche Stadmauerstrasse 25, 91954 Erlangen Bavaria, Germany. During a normal commercial production phase, one wort, designated brew number 120, produced on the 26th of April 1999, containing 286 litres of wort at 11.6° Balling, was separated into two fermenters each containing one hundred and forty-three hectolitres of "pilsner" wort. Both temperature profiles of the fermentations were as per normal for that brewery for that beer type.

Zinc Preparation

Laboratory tests

Mineral zinc, when used, was added in the form of the salt zinc chloride. This salt is used extensively by breweries throughout the World. Zinc measurements on the wort and yeast samples were carried out by atomic absorption spectrometry as per the MEBAK® standard brewery analytical procedures, see e.g. Lutz, A.: Bestimmung, Vorkommen und

31-05-2000

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Verhalten von Kontaminationen durch verschiedene umweltrelevante Spurenelemente in Bereich der Brauerei, Dissertation TU München, (1996), S21 ff.

5 *Preparation of Sacrificial zinc Yeast.*

A sacrificial zinc yeast can be prepared by the person skilled in the art by any method convenient for producing a zinc-rich yeast. Standard methods use the incorporation, absorption and/or adsorption of zinc by the yeast. It should be noted that to implement the present invention, the yeast could be living or dead.

The preparations used in the trials herein reported were produced in some manner as per the following method.

Zinc, at a concentration of between 1,000 and 200,000 ppm (relative to the weight of the yeast or yeast fraction, as measured on a dry weight basis), in the form of zinc sulphate, chloride, acetate, phosphate, or some other appropriate zinc form is added to a live or dead culture of *S. cerevisiae* at a temperature of about 4 to about 40° Celsius (preferably of about 25-32°C) at pH of between about 3.5 to about 7 (preferably about 4.6 to about 6.6), for a period of 1 to 20 or 24 hours so as to allow the culture to incorporate, absorb and/or adsorb the zinc.

Two base dry zinc yeast preparations were used in the trials;

Preparation one contained 10,500 ppm mineral zinc

Preparation two contained 70,000 ppm mineral zinc

Other zinc yeast preparations are also commercially available from Danstar Ferment AG, 20 Alpenstrasse, 6301 ZUG, Switzerland (MEY® Zn 50).

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All the zinc preparations were added to the boiling worts, at the start of boiling.

In summary, the state of zinc in the yeasts and worts in the three laboratory tests and two brewery trials is as follows in :

Table 1

	Test 1	Test 2	Test 3	Trial 1	Trial 2
Wort ppm	0.2	0.06	0.1	0.06	0.1
Fermentation Yeast mg/100g dry	5.9	1.3	4.1	1.3	5

Fermentation Yeast Preparation

A portion of the test yeast, 30 g of the paste, was re-suspended in 250 millilitre of boiled wort and aerated for approximately five minutes by way of a magnetic stirrer. The yeast preparation was then divided into seven equal aliquots and pitched into the appropriate test wort.

Brewery trial one and brewery trial two were conducted similarly.

The yeasts for brewery trial one, and for brewery trial two, were collected from previous fermentations stored as a cream, under conditions of refrigeration, and pitched as per the normal procedure for the brewery.

The yeast used for fermentation was pitched at a level of 1.6 litres of yeast cream per hectolitre of wort (this is normal yeast handling and pitching procedures for this brewery) for brewery trial one, and of 1.8 litres for brewery trial two.

The zinc content of the yeast was measured before pitching.

Experimental Methods

Laboratory Tests

The sample of the brewery wort obtained was divided up into 2.0 litre aliquots.

For laboratory test 1, seven different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6mg. of zinc chloride, which produced a measured increase of 0.28 ppm mineral zinc per litre present in the wort.

Type 2. 40 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.24 ppm zinc present in the wort.

Type 3. 160 mg of sacrificial zinc yeast preparation (at 10,500 ppm zinc) which produced a measured increase of 0.805 ppm zinc present in the wort.

Type 4. 8 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which produced a measured increase of 0.26 ppm zinc present in the wort.

Type 5. 16 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.88 ppm measured additional zinc present in the wort.

For laboratory test 2, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 0.6 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.16 ppm measured additional zinc in the wort.

Type 2. 30 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.8 ppm measured additional zinc in the wort.

Type 3. 160 mg of dry dead brewers yeast, which corresponds to no measured increase in the zinc content of the wort.

Type 4. 0.6 mg of zinc chloride, which corresponds to 0.15 ppm measured additional zinc in the wort.

Type 5. Addition of 80 mg dry dead brewers yeast plus 0.6 mg zinc chloride which corresponds to 0.15 ppm measured additional zinc in the wort.

For laboratory test 3, six different types of aliquots were prepared and additions were made to each as follows :

Type 0. No addition

Type 1. 4.6 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which corresponds to 0.26ppm measured additional zinc in the wort.

Type 2. 160mg of dry dead brewers yeast, which corresponds to 0.01 ppm measured increase of zinc in the wort.

Type 3. 35 mg of sacrificial zinc yeast preparation (at 70,000 ppm zinc) which actually corresponds to a measured increase 1.12 ppm of zinc in the wort.

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Type 4. 2.5 mg. of zinc chloride, which actually corresponds to a measured increase 0.25 ppm measured increase of zinc in the wort.

Type 5. Addition of 160mg dry dead brewers yeast plus 0.6g zinc chloride, which corresponded to a measured increase of 0.29 ppm zinc in the wort.

Each aliquot was boiled for fifteen minutes. The zinc preparations were added at the start of the boil. The boiling vapours were condensed and returned to the respective lot in order to minimise evaporative loss. The wort preparations were sealed, allowed to cool to 8°C then, pitched with the appropriate quantity of yeast.

Brewery Trials

For brewery trial 1, as for brewery trial 2, one fermenter received the equivalent of 0.30 ppm of additional zinc whilst the other received nothing.

Fermentation

Laboratory Tests one, two and three

Fermentation was carried out in a constant temperature room at approximately 10°C until a density of 3.6°P had been achieved. This is normal for beers that are transferred to lager under conditions of refrigeration so that secondary fermentation and maturation can take place.

Brewery Trials

Fermentation was carried under the standard temperature programme for that particular wort type. The standard and test worts were subject to the same profile.

5

Measurements

Laboratory tests one, two and three.

10

Samples were drawn from the wort prior to pitching with yeast and the zinc content was measured.

Fermentation progress was measured by a standard brewing densitometer and recorded in degrees Plato after compensation for temperature effects.

15

At the beginning of fermentation zinc determinations were carried out on the untreated and treated worts and the pitching yeast. Determinations had previously been carried out on the zinc yeast preparations.

The finished beer in all three trials was analysed using a SCABA® "automatic beeranalyser" from PERSTOP ANALYTICAL, SWEDEN, for alcohol concentration as expresses in volume per volume.

20

Brewery trials

During fermentation samples were drawn at regular intervals, and progress of the fermentation, was measured by the drop in density of the wort, as expressed in degree Balling.

25

Results and Conclusions

Laboratory Tests 1, 2, and 3

- 5 The time taken, for each fermentation to achieve 3.6 degree Plato, which is judged to be when primary fermentation is complete and the alcohol concentrations after two hundred and forty hours of fermentation, are detailed below in table 2 for laboratory test 1, in table 3 for laboratory test 2, and in table 4 for laboratory test 3.

10 Table 2

In Addition		Zinc Chloride	Zinc Yeast	Zinc Yeast	Zinc Yeast	Zinc Yeast
Zinc Conc. ppm	0.2	0.48	0.44	1.05	0.46	1.08
Hours to achieve 3.6°P	186	168	150	127	110	110
Hours difference from standard	0	-14	-36	-59	-76	-76
% of standard fermentation time	100	90	81	68	59	59
Alcohol by Volume	4.74	4.92	5.05	5.3	5.26	5.3

Table 3

	0	1	2	3	4	5
Addition		Zinc Yeast	Zinc Yeast	Dead yeast	Zinc Chloride	Dead Yeast + zinc chloride
Zinc Conc. ppm	0.06	0.22	0.86	0.06	0.21	0.21
Hours to achieve 3.6°P	175	153	148	186	156	163
Hours difference from standard	0	-20	-24	+10	-14	-2
% of standard fermentation time	100	87	84	106	89	93
Alcohol by Volume	4.86	5.02	5.05	4.93	4.91	4.96

Table 4

	0	1	2	3	4	5
Addition		Zinc Yeast	Dead Yeast	Zinc Yeast	Zinc Chloride	Dead Yeast + Zinc chloride
Zinc Conc. ppm	0.1	0.36	0.11	1.22	0.35	0.39
Hours to achieve 3.6°P	182	162	180	158	177	168
Hours difference from standard	0	-20	-2	-24	-5	-14
% of standard fermentation time	100	89	99	87	97	92
Alcohol by Volume	5.78	5.86	5.81	5.86	5.6	5.74

Conclusions

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1. In all fermentation tests where sacrificial zinc yeast was added, fermentation speed was improved in comparison to the standard wort, wort with added zinc chloride, and, when tested, wort with added dead yeast, and wort with added zinc chloride plus dead yeast.

10

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2. In all fermentation tests where sacrificial zinc yeast was added, the standard specific gravity, designated for onward processing of the beer for lagering, was achieved faster than the standard wort, wort with added zinc chloride, and, when tested, wort with added dead yeast, and wort with added zinc chloride plus dead yeast. The time to achieve this degree of fermentation attenuation was at least twenty hours and as great as seventy-six hours less than the standard.
3. Against the test where zinc chloride was added, the sacrificial zinc yeast trials achieved the standard fermentation attenuation at least six and as great as sixty-two hours sooner.
4. Where the zinc addition was at a similar level from mineral zinc (zinc chloride), and biological zinc (sacrificial zinc yeast), the sacrificial zinc yeast experiments were measurably and significantly faster.
5. In all fermentation tests where sacrificial zinc was added, the final concentration of alcohol produced after ten days, was greater than the standard wort, the wort containing zinc chloride, and when tested, the wort containing inactivated yeast and the wort containing zinc chloride plus inactivated yeast.

Brewery trial 1 and trial 2

Data collected from the brewery fermentation trial number 1 and 2 are displayed in the below table 5. Graphical representation of these data are displayed in figure 1 for brewery trial 1, and in figure 2 for brewery trial .

Table 5

	Trial 1 (primary attenuation limit 3.8)		Trial 2 (primary attenuation limit 3.6)	
Fermentation days	0-Wort	Zinc Yeast	0-Wort	Zinc Yeast
0	11.6	11.6	11.5	11.5
1	10.8	9.6	10.5	10.5
2	9.5	7.8	9	7.5
3	8.8	6	7	6
4	7.6	4.3	5.2	3.5
5	6.7	3.9	4.2	2.1
6	5.7	3.4	3.5	2
7	4.8	2.8	2.8	1.9
8	4.4	2.7	2.6	1.8
9	4	2.7	2.2	1.8

Conclusions

1. The fermentation containing sacrificial zinc yeast was faster than the standard untreated wort.
2. In the fermentation trials where sacrificial zinc yeast was added, the standard specific gravity, 3.8 and 3.6 degrees Balling respectively, designated for onward processing of the beer for lagering was achieved one hundred and forty hours sooner (trial 1), and forty eight hours sooner (trial 2), than the standard untreated wort.

The two beers produced were tasted, by a party of professional brewers and others with professional expertise in beer tasting. The trial beer was judged to be at least as good as the standard and was preferred by many.

The tasters noted that both test beers were particularly lower in "sulphitic" character. This is of particular importance and significance for not only does it indicate a "cleaner" beer it indicates a possibility of advancing the maturation process and thus reducing lager times and costs. At the cellular level, zinc addition according to the invention can show the following stimulatory effects: stabilising proteins and membrane systems, acting as a catalytic centre of essential enzymes (*e.g.* alcohol dehydrogenase, aldolase and acetaldehyde dehydrogenase), enhancing riboflavin synthesis, activating acid and alkaline synthesis, stimulating the uptake of maltose and maltotriose.

EXAMPLE 2 : Residual sugar analysis

After two hundred and forty hours of fermentation of laboratory test the residual sugars in all the beers of test 1 were analysed by gas liquid chromatography. The results are detailed in the below table 6.

Table 6

Experiment	0	1	2	3	4	5
Sugars as mg/100ml		Zinc Chloride	Zinc Yeast	Zinc Yeast	Zinc Yeast	Zinc Yeast
Glucose	0	0	0	0	0	0
Fructose	0	0	0	0	0	0
Sucrose	0	0	0	0	0	0
Maltose	0.69	0.47	0	0	0	0
Maltotriose	0.15	0.09	0	0	0	0
Total	0.84	0.56	0	0	0	0

Conclusions

It is clear from the data presented above that the inclusion of sacrificial zinc yeast permits the fermentation to progress to absolute dryness. That is to say there are no fermentable sugars left in the beer. This is highly significant as it will permit brewers to produce beers free of residual fermentable sugars much quicker than at present. The technical features of the process according to the invention thus lead to more alcohol produced, thereby giving an economic advantage.

EXAMPLE 3 : Quality and quantity of secondary flavour compounds.

After the completion of the fermentations in laboratory test 2 (see the above example 1) the beer was subject G.L.C. analysis to see if the accelerated fermentations affected the quality and quantity of secondary flavour compounds. The results are detailed in the below table 7.

Table 7

Mg/L	0	1	2	3	4	5
	0	Zinc Yeast	Zinc Yeast	Dead Yeast	Zinc chloride	Dead yeast + Zinc chloride
Diacetyl	0.22	0.27	0.29	0.22	0.24	0.24
Pantadione 2.3	0.21	0.25	0.26	0.22	0.22	0.23
Acetadehyde	24.2	25.6	27.9	25.9	24.2	25.5
Ethyl acetate	28.7	25.2	30.9	27.3	28.7	27.8
i-Butanol	7.6	7.5	8.1	7.4	7.6	8
n-Propanol	10.2	10.4	11.4	10.7	10.4	11.2
Amyl acetate	2.8	2.5	3.2	2.7	2.9	2.8
Amyl alcohol	52.1	49.3	52.7	50.9	51.4	52

Conclusions

5 It is evident from the results above that accelerating the fermentation, by way of sacrificial zinc yeast, has no significant negative effect on the major, secondary, organoleptically active metabolites. Such a negative effect has indeed not been observed in any of the tests and trials performed (see the above example 1). This is particularly striking effect of the process of the invention which allows an accelerated fermentation without negatively affecting the alcohol profile of the product. These
10 conclusions on the quality of the beer produced according to the invention were further confirmed by blind taste panels.

This is a significant finding as it allows a standard beer to be produced at a faster, and therefore cheaper rate.

15 It will be apparent to those skilled in the art that the process of the present invention which comprises the use of a mineral-rich yeast, and in particular of a zinc-rich yeast, as a fermentation nutrient is a very valuable technical contribution to additive-free brewing. It will also be apparent that the foregoing examples have been for purposes of illustration, and
20 that a number of changes and modifications can be made without departing from the scope of the invention. The present invention illustrated with a zinc-rich yeast can thus be implemented without undue burden with a yeast rich in any mineral or combination of minerals appropriate to a yeast growth enhancement process, e.g. magnesium,
25 manganese.

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CLAIMS

1.- Process for alcoholic fermentation comprising the use of a fermentation micro-organism, characterized in that it also comprises the use of at least one mineral-rich or mineral-enriched yeast as a nutrient source for said fermentation.

2.- Process according to claim 1, characterized in that said at least one mineral-rich or enriched yeast belongs to the *Saccharomyces* genera or to the *Kluyveromyces* genera.

3.- Process according to any one of claim 1 or 2, characterized in that said at least one mineral-rich or mineral-enriched yeast is prior to use obtainable by adding 1,000 to 200,000 ppm (relative to the weight of the yeast, as measured on a dry weight basis) of a salt of said mineral to a live culture of said micro-organism at a temperature of 4-40°C at a pH of between 3.5 to 7.0, for a period of 1-24 hours, so as to allow said micro-organism to incorporate said mineral.

4.- Process according to claim 3, characterized in that said temperature is in the 25-32°C range.

5.- Process according to claim 3, characterized in that said pH is in the 4.6-6.6 range.

6.- Process according to claim 3, characterized in that said time period is in the 2-16 hour range.

7.- Process according to any one of claims 3-6, characterized in that said salt is chosen among the group consisting of acetate, caprylate, carbonate, chloride, chromate, gluconate, iodate, lactate, oleate, oxide,

perchlorate, peroxide, phosphate, salicylate, sulphate, sulphide, tartarate or valerate.

8.- Process according to any one of claims 3-7, characterized in that said mineral incorporation corresponds to an absorption and/or an
5 adsorption.

9.- Process according to any one of claims 1 to 8, characterized in that said mineral is a metal capable of altering the metabolism of said fermentation.

10.- Process according to any one of claims 1 to 9, characterized in
10 that said mineral is chosen among the group consisting of zinc, magnesium and manganese.

11.- Process according to any one of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast contains before being used a concentration in said mineral ranging from
15 1,000 to 200,000 ppm .

12.- Process according to any one of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a living form and a dead form.

20 13.- Process according to any one of the preceding claims, characterized in that said at least one mineral-rich or enriched yeast is used under a form chosen among the group consisting of a dry form, a liquid form, a frozen form, a freeze-dried form, a paste, a powder.

14.- Process according to any one of the preceding claims,
25 characterized in that said at least one mineral-rich or enriched yeast is used by directly adding it at at least one step of said fermentation process.

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15.- Process according to claim 14, characterized in that said addition is performed directly into at least one element selected from the group consisting of a fermenter, a boiling vessel, any vessel between the two, a fermentation micro-organism holding vessel, a fermentation micro-organism holding vessel.

16.- Process according to any one of the preceding claims, characterized in that said yeast is used at such a quantity and/or at such a concentration in said mineral that it leads to an increase of at least 0.05 ppm of the mineral content of the substrate of said fermentation.

17. - Process according to any one of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of beer.

18.- Process according to any one of the preceding claims, characterized in that said alcoholic fermentation can lead to the production of an alcohol chosen among the group consisting of whisky or sake as well as fruit, sugar or honey based fermentations, such as wine, brandy, cider, fruit wines, mead, rum, tequila, industrial alcohols, potable alcohols.

19.- Use of a fermentation micro-organism and of at least one mineral-rich or mineral-enriched yeast as a nutrient source in the production of an alcohol by fermentation.

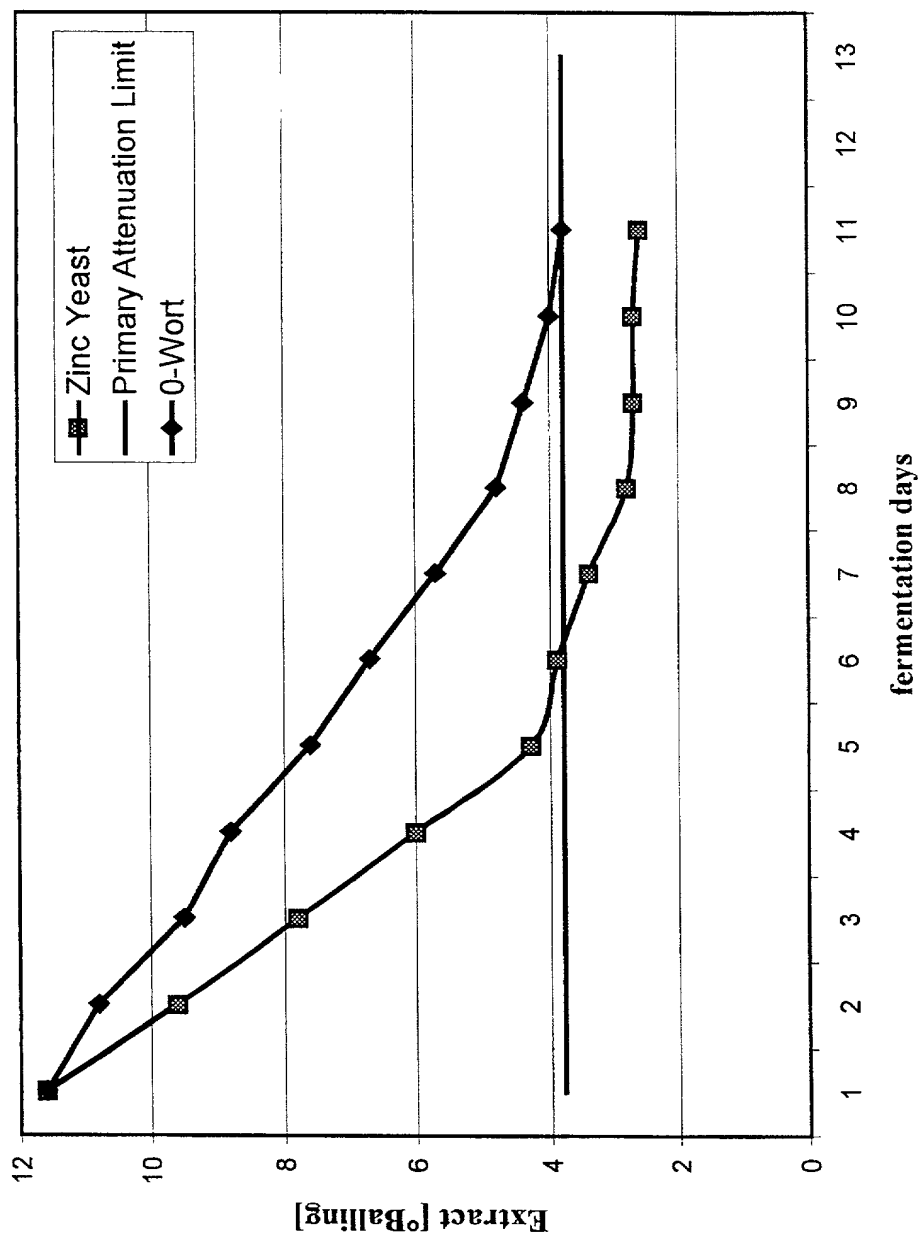


Figure 1

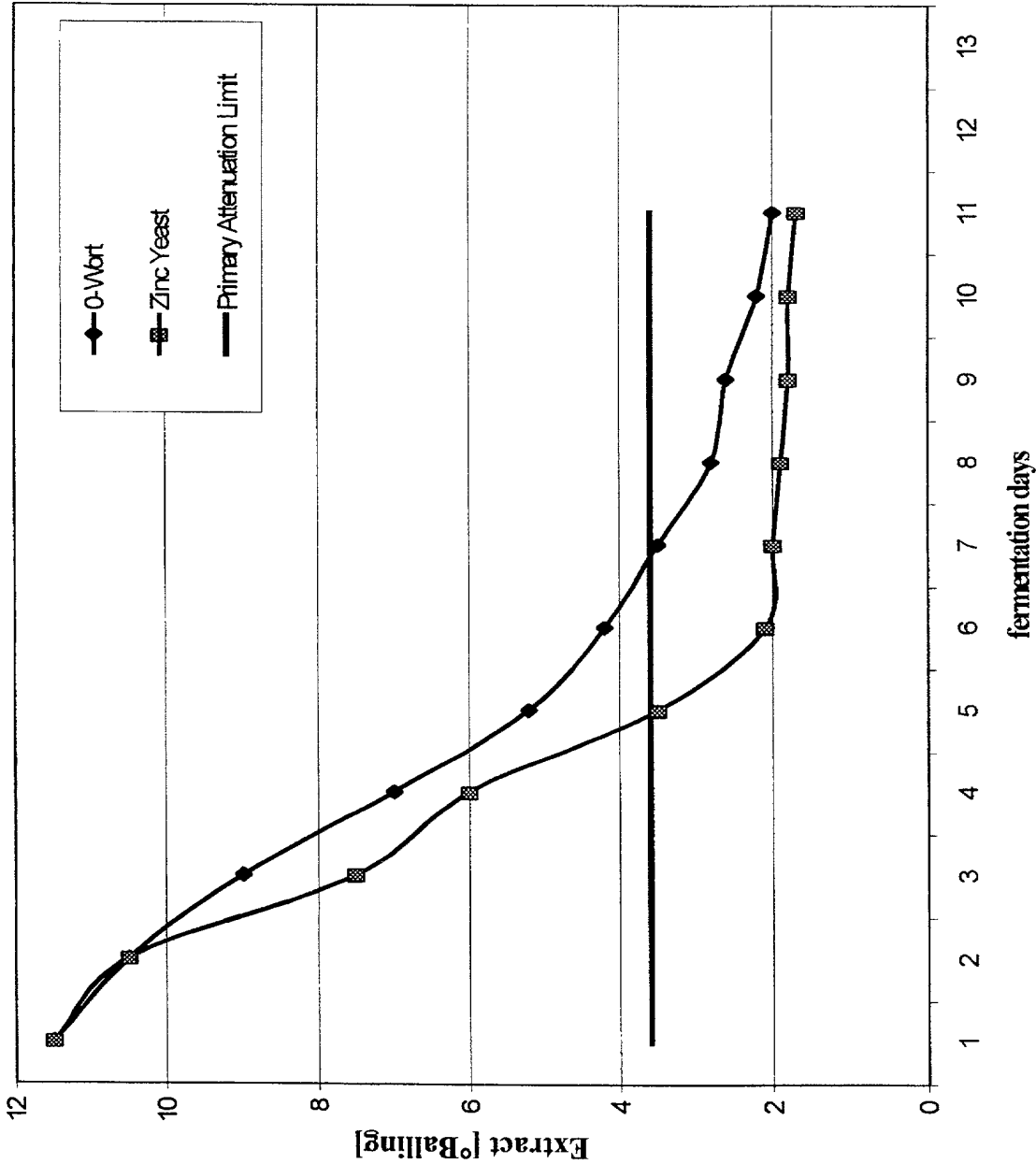


Figure 2

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Improved process for alcoholic fermentation

the specification of which

- ☐ is attached hereto.
☐ was filed on _____ as Application Serial No. _____
 and was amended on _____.
☒ was described and claimed in PCT International Application No. PCT/IB99/00924
 filed on May 21, 1999 and as amended under PCT Article 34 on July 11, 2000

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
Great Britain	98 11103.2	May 23, 1998	Yes/No Yes
			Yes/No
			Yes/No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as

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defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,462, Karen L. Elbing, Reg. No. 35,238, and Kristina Bieker-Brady, Reg. No. 39,109.

Address all telephone calls to: 617/428-0200.

Address all facsimile messages to: 617/428-7045.

Address all correspondence to: Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
James McLaren	France <i>JRX</i>	La Bourdeneuve de Lavat 32700 Lectoure- France-	British
Signature: <i>James McLaren</i>			Date: Oct. 12, 2000

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Signature:			Date:

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Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Signature:			Date: